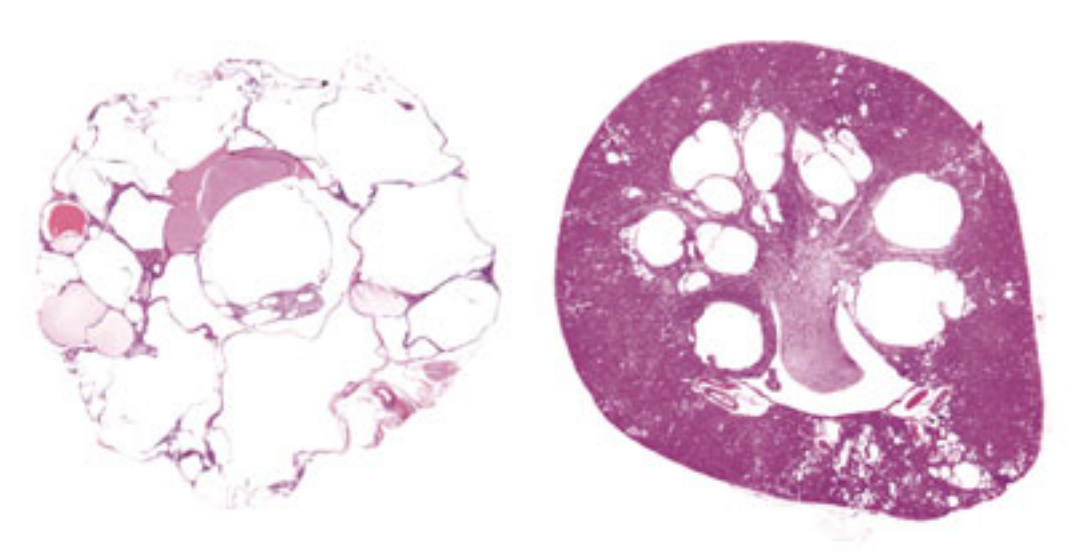


Left: Histologic section taken from kidneys of mice that develop kidney disease similar to polycystic kidney disease (PKD) in humans. Right: Section of a cystic kidney taken from a rat model of PKD. Scientists use animal models of diseases to study progression and evaluate potential new therapeutic approaches. Images courtesy of Dr. Vicente Torres and reprinted from Torres *et al*. *Nat Med* 10: 363-364 (2004) and Gattone *et al*. *Nat Med* 9: 1323-1326 (2003).

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the U.S. They afflict millions of Americans, including children and young adults. The NIDDK is committed to enhancing research to understand, treat, and prevent these diseases.

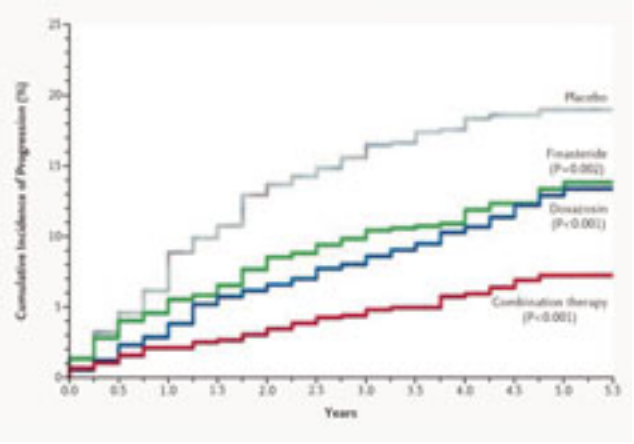
Normal, healthy kidneys filter toxins from the blood, concentrating them in urine so that they may be ultimately excreted from the body. In people with chronic kidney disease, the function of these life-sustaining organs is impaired. Kidney disease may progress to irreversible kidney failure, also known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. Conservative estimates find that 4.5 percent of American adults 20 years of age and older—about eight million adults—have substantially impaired kidney function. The leading cause of kidney disease is diabetes, with hypertension (high blood pressure) the second-leading cause. The recent increases in obesity and type 2 diabetes in the U.S., if left unchecked, will have grave implications in several years, as more people begin to develop renal complications of diabetes.

Racial minorities, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease. African Americans are four times more likely and American Indians are twice as likely to develop kidney failure than are non-Hispanic whites. Hispanics have a significantly increased risk for kidney failure, as well.

The U.S. has seen an enormous increase in the number of people with ESRD. The NIDDK-supported United States Renal Data System (USRDS), a nationwide database of kidney disease information, reports that over 100,000 people developed ESRD in 2002, the most recent year for which statistics are available. Additionally, over 400,000 patients were living with the disease at the end of that year, with more than 300,000 receiving dialysis and over 120,000 with a functioning kidney transplant. These numbers have doubled since 1990 and are expected to nearly double again by 2010.

The NIDDK supports a significant body of research aimed at increased understanding of the biology underlying chronic kidney disease. The chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease; the underlying mechanisms leading to progression of kidney disease to ESRD; and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. Areas of focus include diseases that collectively account for more than half of all cases of treated ESRD. Of special interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related glomerular diseases, including IgA nephropathy and the hemolytic uremic syndrome. A major new educational outreach effort is the National Kidney Disease Education Program, which is designed to raise awareness among patients and physicians about the problem of kidney disease and steps that should be taken to treat chronic kidney disease and prevent kidney failure.

Urologic diseases affect men and women of all ages, result in significant health care expenditures, and—if misdiagnosed or improperly treated—may lead to substantial disability and impaired quality of life. The NIDDK's urology research portfolio includes basic and clinical research on the normal and abnormal development, structure, and function of the genitourinary (GU) tract. The NIDDK also supports studies of a number of noncancerous urologic



The MTOPS clinical trial found that combination therapy with two drugs that act through different mechanisms was more effective at preventing progression of benign prostatic hyperplasia (BPH) than either drug alone. The graph shows the cumulative incidence of progression in men with BPH who received either a sugar pill (placebo, grey line); an inhibitor of the enzyme 5-alpha reductase (finasteride, green line); a beta blocker (doxazosin, blue line); and finasteride and doxazosin together (red line). For more information, see page 74.

Image reprinted from McConnell *et al*. *New Engl J Med* 349: 2387-2398 (2003). Copyright © 2003 Massachusetts Medical Society. All rights reserved.

diseases, include benign prostatic hyperplasia, prostatitis, urinary tract infections, urinary tract stone disease, interstitial cystitis, urinary incontinence, and congenital anomalies of the GU tract.

Benign prostatic hyperplasia, or BPH, is a serious condition that is especially common among older men. Almost one-half of men over age 70 report lower urinary tract symptoms that are consistent with a diagnosis of BPH. Prostatitis—chronic inflammation of the prostate gland—is a painful condition that accounts for a significant percentage of all physician visits by young and middle-aged men for complaints involving the genital and urinary systems. The NIDDK is committed to enhancing research to understand, treat, and prevent these common and troubling disorders.

Infections of the urinary tract are extremely common in women, and many women suffer repeated urinary tract infections (UTIs). In 2000, UTIs and cystitis accounted for over nine million physician visits. Interstitial cystitis (IC) is a debilitating, chronic, painful bladder disease that has been estimated to affect as many as 847,000 American adults, over 90 percent of whom are women. Millions of Americans, most of them women, suffer from urinary incontinence. For both men and women, kidney stones, a condition formally known as urinary tract stone disease, accounted for over 2.2 million physician visits in 2000. In children, one of the most common causes of kidney failure, vesicoureteral reflux, occurs in an estimated 1-to-2 percent of newborns. In fact, abnormalities of the GU tract are the most common birth defects.

To address these and other urologic problems, the NIDDK's urology research efforts support basic, applied, and clinical research in prostate function and prostate diseases; diseases and disorders of the bladder; male sexual dysfunction; urinary tract infections; urinary tract stone disease; and pediatric urology, including developmental biology of the urinary tract. A research emphasis of the urology program is the study of chronic inflammatory disorders of the lower urinary tract.

The NIDDK's hematology research program uses a broad approach to enhance understanding the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, and thrombocytopenia. The Institute is also keenly interested in the basic biology and genetic regulation of stem cells, especially adult hematopoietic stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the Institute's hematology research program is the development of improved iron chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

ADVANCES IN KIDNEY DISEASE RESEARCH

A Potential New Therapy for Polycystic Kidney Disease (PKD): PKD and other inherited cystic kidney diseases frequently cause kidney failure and death, often in children. There are no effective treatments. One characteristic common to several of these disorders is an elevated level of cyclic adenosine monophosphate (cAMP) in the kidneys. Within cells, cAMP transmits messages that affect their growth and function; abnormally high levels of cAMP in certain kidney cells are thought to contribute to cyst formation. Researchers treated animal models of the two predominant forms of human PKD and another cystic kidney disease using a chemical, OPC31260, which lowers cAMP production in the kidneys. The treatment halted disease progression, and in some cases resulted in improvement. OPC31260 and similar compounds are currently undergoing testing in human clinical trials for treatment of other diseases and so far appear to be safe. Thus, drugs of this class are promising candidates for phase I clinical trials to treat patients with PKD.

Torres VE, Wang X, Qian Q, Somlo S, Harris PC, and Gattone II, VH. Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat Med* 10: 363-364, 2004.

Gattone II, VH, Wang X, Harris PC, and Torres VE. Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat Med* 9: 1323-1326, 2003.

Impact of Chronic Kidney Disease on Cardiovascular Health: Of the estimated eight million Americans with chronic kidney disease,[1] more than 400,000 have end-stage renal disease, or ESRD, with over 300,000 requiring dialysis to live.[2] ESRD patients are known to have very high rates of cardiovascular disease (CVD), which kills about half of them. However, until recently, it was unknown to what degree less serious chronic kidney disease predisposes patients to develop CVD. The Modification of Diet in Renal Disease clinical trial provided strong evidence that kidney function can be reliably estimated by measuring the amount of a compound called creatinine in a patient's blood, and performing a calculation that also includes variables such as the person's size and sex. Now, researchers have built upon that finding by examining the results of creatinine tests from more than one million patients to assess kidney health and look for correlations with cardiovascular outcomes such as heart attacks. The researchers found a very clear pattern: the poorer a patient's kidney function, the more likely he or she was to develop CVD. Armed with the knowledge that kidney health is a predictor of CVD, health care providers can now determine that some of their patients are at risk, and may be more likely to benefit from earlier, more aggressive cardiovascular treatment than might otherwise have been prescribed.

Go AS, Chertow GM, Fan D, McCulloch CE, and Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 351: 1296-1305, 2004.

Hostetter TH. Chronic kidney disease predicts cardiovascular disease. *N Engl J Med* 351: 1344-1346, 2004.

Statistical References

[1] Coresh J, Astor BC, Greene T, Eknoyan G, and Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 41: 1-12, 2003.

[2] 2004 USRDS Annual Data Report Atlas. United States Renal Data System, 2004. (*http://www.usrds.org/atlas.htm*)

Pinpointing the Location of Adult Kidney Stem Cells: The cells of a healthy adult kidney rarely divide to make new copies of themselves. However, kidneys retain a limited capacity for self-repair in case of injury. That repair requires replacing damaged cells of multiple types. This is the kind of task the body relegates to stem cells, which by definition can divide and differentiate into multiple cell types. Recent research using rodents has determined that stem cells capable of forming new kidney cell types are largely or entirely confined to a small portion of the kidney called the "renal papilla." Further research

can now proceed to determine whether these cells can form any kidney cell type, or just a subset of them. Even more importantly, researchers will be seeking the specific signals that trigger kidney stem cells to form each cell type. With this knowledge, it may one day be possible to stimulate patients' innate ability to heal their kidneys, thereby reducing the need for dialysis and kidney transplantation.

Oliver JA, Maarouf O, Cheema FH, Martens TP, and Al-Awqati Q. The renal papilla is a niche for adult kidney stem cells. *J Clin Invest* 114: 795-804, 2004.

Kidney Disease Clinical Research: The NIDDK recognizes the importance of encouraging the development of novel ideas for clinical interventions related to kidney disease. For the past two years, the NIDDK has sponsored a pilot program aimed at providing supplemental funding to NIDDK-supported investigators to encourage them to undertake such studies. The "Kidney Disease Clinical Studies Initiative" is an outgrowth of a task force meeting convened in March 2002 by the NIDDK and the Council of American Kidney Societies (CAKS). Since the inception of this initiative, the NIDDK has seen growing demand from the research community for this funding, and has developed new funding mechanisms for research concept development and ancillary studies. The NIDDK anticipates holding a follow-up meeting with the kidney research community sometime in 2005 or 2006 to discuss ways of strengthening this program. Also, plans for a program of small planning grants for clinical studies have grown out of the experience with supplemental awards.

TREATMENT STRATEGIES FOR BENIGN PROSTATIC HYPERPLASIA

Combination Therapy for Benign Prostatic Hyperplasia: Benign prostatic hyperplasia, or BPH, is a condition that affects an estimated nine percent of men 30 years of age and older. Prevalence increases significantly in middle age, with the majority of cases reported in men age 55 and older. BPH can result in frequent urination, inability to urinate, and urinary tract infections. For many years, surgery was the only viable treatment option, although new drug therapies have recently emerged from research studies. The NIH launched the Medical Therapy of Prostatic Symptoms (MTOPS) clinical trial to assess the safety and efficacy of different interventions on BPH symptoms and progression. Study participants were divided into four groups, and received either placebo (sugar pill), one of two Food and Drug Administration-approved medications for BPH with different mechanisms of action, or the two drugs in combination. The study followed participants for an average of five years and the results were striking. Although each drug was effective when used alone (the risk of BPH progression was reduced by 39 percent with one and by 34 percent with the other), the combination drug therapy reduced the risk of BPH progression by 67 percent compared to placebo. The MTOPS trial conclusively demonstrated that combination therapy is safe, and is the most effective treatment for men with symptomatic BPH.

McConnell JD, Roehrborn CG, Bautista OM, Andriole GL, Jr., Dixon CM, Kusek JW, Lepor H, McVary KT, Nyberg Jr, LM, Clarke HS, Crawford ED, Diokno A, Foley JP, Foster HE, Jacobs SC, Kaplan SA, Kreder KJ, Lieber MM, Lucia MS, Miller GJ, Menon M, Milam DF, Ramsdell JW, Schenkman NS, Slawin KM, and Smith JA for the Medical Therapy of Prostatic Symptoms (MTOPS) Research Group. The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. *N Engl J Med* 349: 2387-2398, 2003.

Despite advances in the medical treatment of BPH, some men will ultimately require surgery to alleviate their symptoms. For many years, transurethral resection of the prostate (TURP) has been the standard surgical therapy for this condition. With TURP, an instrument called a resectoscope is inserted up the urethra. The physician uses the resectoscope's wire loop to remove the tissue causing the obstruction of the urethra, thereby alleviating the discomfort and urinary urgency associated with BPH.

Over the past decade, a number of technical innovations have furthered the development of new

surgical treatments that aim to achieve the same long-term outcomes of TURP, but with less morbidity, lower costs, in-office treatment or shorter hospital stays, and more rapid recovery. These new, "minimally-invasive" surgical approaches include laser therapy, hyperthermia and thermotherapy, transurethral electrovaporization, microwave therapy, and transurethral needle ablation. Furthermore, newer techniques are appearing regularly. However, published reports on the outcomes of these minimally-invasive therapies are highly variable in their quality, and rigorously conducted long-term, multicenter randomized clinical trials have only rarely been conducted. To assess the long-term safety and effectiveness of these new therapies, the NIDDK has launched the MIST clinical trial. MIST, "Minimally Invasive Surgical Therapy for BPH," will evaluate the safety and effectiveness of transurethral needle ablation (TUNA) and transurethral microwave therapy (TUMT), procedures whereby radio waves or microwaves, respectively, are used to heat and destroy obstructing prostate tissue; and combined medical therapy with alfuzosin, an alpha blocker, and finasteride, an alpha-reductase inhibitor in men with BPH. The results of this trial will help to provide both physicians and patients with the knowledge needed to make the most appropriate choices for long-term management of BPH.

OPPORTUNITIES IN WOMEN'S UROLOGIC HEALTH

Urinary Incontinence: More than 13 million people in the U.S.—men and women of all ages—experience urinary incontinence. Women experience incontinence twice as often as men. Pregnancy and childbirth, menopause, and the structure of the female urinary tract account for this difference. Incontinence in women usually occurs because of problems with muscles that help to hold or release urine. One kind of urinary incontinence is stress urinary incontinence, which is the accidental leakage of urine during activities such as coughing, laughing, sneezing, or lifting heavy objects. Another type, urge incontinence, is the leakage of large amounts of urine at unexpected times, including during sleep.

To address the problem of urinary incontinence in women, the NIDDK, along with the National Institute of Child Health and Human Development and the NIH Office of Research on Women's Health (ORWH), supports a Urinary Incontinence Treatment Network (UITN). The Network is a group of urologists and urogynecologists who are investigating possible new treatments. The Network has begun a clinical trial comparing two surgical treatments for stress and mixed incontinence—the "Stress Incontinence Surgical Treatment Efficacy Results" (SISTER) trial. The SISTER study is comparing the long-term outcomes of two commonly performed surgeries for the treatment of stress urinary incontinence. A second clinical trial planned by the Network is focused on treating women with pure or predominantly urge incontinence. This trial, the "Behavior Enhances Drug Reduction of Incontinence" (BE-DRI) trial, will compare effects of two interventions—drug therapy alone and combination drug therapy and behavioral treatment—on the frequency of urinary incontinence and success in withdrawing patients from drug therapy.

In another effort aimed a treating incontinence, the NIDDK has recently funded the "Program to Reduce Incontinence by Diet and Exercise" (PRIDE) study, which will evaluate the impact of weight loss, from a behavioral program, on urinary incontinence in overweight and obese women. About 300 overweight women ages 30 or older will participate in this clinical trial.

Interstitial Cystitis: Interstitial cystitis (IC) is a chronic bladder disease characterized by pelvic pain and increased frequency and urgency in urination. These symptoms can be quite debilitating, interfering with a patient's ability to work, go out, and enjoy life. While the precise number of persons affected is unknown, as many as 847,000 American adults may suffer from IC; however, 90 percent of reported cases occur in women. The causes of IC are as yet unknown. Current treatments

for symptoms are not effective in all patients, and there is no cure. The NIDDK is supporting clinical and basic research investigations on several fronts to understand the causes of IC, to develop and test more effective treatments, to develop better diagnostic tools, and, ultimately, to develop a cure for this disease.

In October 2004, the NIDDK held a meeting of the more than 20 grantees who received research funding through a recent Request for Applications (RFA) for "Basic Research in Interstitial Cystitis." The group discussed ongoing work and heard descriptions of encouraging results and future plans for work on a promising biomarker for IC, antiproliferative factor (APF). To build on this discovery, the NIDDK is currently developing a translational research initiative that will accelerate efforts to validate APF's usefulness as a diagnostic tool for IC. The NIDDK plans to support another meeting of the investigators in 2005; this meeting will help continue the critically important cross-fertilization of ideas among researchers in the field, and will also help guide the Institute's decisions regarding support for larger meetings of IC investigators in the future.

In Fall 2004, the NIDDK initiated its new Interstitial Cystitis Awareness Campaign. This campaign will reach out to two target audiences: healthcare professionals, especially urologists, and American women between the ages of 25 and 50 who may have IC. Outreach materials for this campaign include information on symptoms, diagnostic protocols, treatment strategies and research for IC patients. The National Kidney and Urologic Diseases Information Clearinghouse is the primary distribution channel for campaign materials. In developing this important awareness campaign for patients and healthcare practitioners, the NIDDK received input from a patient-based advocacy group for IC through its participation in an *ad hoc* coordinating panel for the Clearinghouse.

Efforts to inform physicians and the public about IC will be significantly enhanced when there is a consistent and clinically useful definition. Some progress has been made in identifying common clinical symptoms among some IC patients. The NIDDK anticipates that recent efforts to review the rapidly evolving science surrounding IC will lead to a better scientific basis with which to approach the development of a consensus definition for IC. Examples of these efforts include: the work of the IC Epidemiology Task Force convened by NIDDK in October 2003; the work of the NIDDK Subcommittee on the Diagnosis of Interstitial Cystitis and Painful Bladder Syndrome, which presented its recommendations at the 2003 IC research symposium co-sponsored by the Interstitial Cystitis Association and is currently preparing them for publication; and the previously-mentioned planned initiative to validate APF as a diagnostic tool in larger studies.

Urinary Tract Infections: Urinary tract infections are a serious health problem affecting millions of people each year. Infections of the urinary tract are common; only respiratory infections occur more frequently. In 1997, urinary tract infections (UTIs) accounted for about 8.3 million visits to physicians. Most UTIs are caused by the common *Escherichia coli* (*E. coli*) bacteria. A UTI begins when bacteria enter the bladder, provoking an immune response and the sloughing off of bladder cells into the urine in the body's attempt to rid itself of offending bacteria.

Women are especially prone to UTIs for reasons that are poorly understood. One woman in five develops a UTI during her lifetime. Many women suffer from frequent UTIs: nearly 20 percent of women who have a UTI will have another, and 30 percent of those will have yet another. Recent research suggests that one factor behind recurrent UTIs may be the ability of bacteria to attach to cells lining the urinary tract. Bacteria can form a protective film on the inner lining of the bladder in mice, effectively "hiding out" from the immune system.

KIDNEY AND UROLOGIC DISORDERS OF CHILDHOOD

Chronic Kidney Disease in Children: Chronic kidney disease (CKD) is associated with numerous metabolic problems that can have significant negative impacts on the overall well-being of children with the disease. Growth impairment is one well-documented consequence of CKD in children, but there is less information about other developmental problems, such as impaired brain development and risk of cardiovascular disease. To address this lack of knowledge, in late 2002 the NIDDK, in collaboration with the National Institute of Neurological Disorders and Stroke (NINDS) and the National Institute of Child Health and Human Development (NICHD), solicited research applications for a prospective epidemiological study of children with CKD. In 2004, the National Heart, Lung, and Blood Institute (NHLBI) joined this initiative. Currently, the NIH supports two clinical centers and a data coordinating center that lead this study. The primary goals of this study are to determine the risk factors for the decline in kidney function in children with CKD; identify the risk factors for and incidence of impaired neurocognitive development and function; determine the prevalence of risk factors for cardiovascular disease; and examine the long-term effects of growth failure and its treatment in these children. The information obtained from this study will establish important natural history and outcome measures that will guide future intervention or prevention trials of pediatric CKD.

Research into Malformations of the Urinary Tract: During normal urination, the bladder contracts and expels urine through the urethra and out of the body. When this occurs, a valve-like structure at the back of the bladder closes to prevent urine from traveling back up the ureters toward the kidneys. However, in children with vesicoureteral reflux (VUR), this valve is either malformed or does not function properly, and some urine backs up into the ureters, possibly as far as the kidneys. This reflux can expose the kidneys to bacteria present in the urine, leading to kidney infection and possible kidney damage. Untreated reflux to both kidneys can, in the most severe instances, result in kidney failure, requiring dialysis or kidney transplantation. Because VUR is a congenital malformation, it is often first identified in children. Although some children will see their disease correct itself as they grow, others require surgery to address this condition. The NIDDK is currently acting on recommendations from a May 2003 meeting of the Vesicoureteral Reflux Task Force, which focused on the potential for conducting a randomized, controlled clinical trial in diagnosed children. The group identified several aspects of disease progression and treatment that are poorly understood and about which a clinical trial could provide important insights. In July 2004, the NIDDK issued a Request for Applications (RFA) for pediatric nephrology/urology clinical centers for the design and conduct of treatment trials and studies in affected children. The primary goals for this program are to study disease progression in a cohort of 600 children with mild to moderate disease and to determine which interventions are most beneficial. The NIDDK anticipates that planning for a clinical trial will begin in late 2005.

Other Studies of Congenital Urinary Tract Anomalies: The GU tract is the organ system most commonly affected by congenital birth defects, and many reports suggest that some of the birth defects may be increasing in incidence. Research into the normal development of the genitourinary tract is limited by a lack of cell-specific markers for key cell lineages within the developing GU tract, incomplete understanding of the normal three dimensional cellular structure of the major organs of the GU tract, incomplete understanding of the morphogenetic events that occur during organogenesis, and the lack of a detailed integrative database to assimilate complex temporal and spatial expression data. In late 2003, the NIDDK and NICHD solicited research applications for the development of a "Murine (mouse) Atlas of Genitourinary

Development." These projects would contribute to an anatomical and gene expression atlas of the developing mouse GU tract. When complete, the data from this atlas will greatly enhance scientists' understanding of events that occur during organogenesis (organ development). The atlas may lead to strategies for new and innovative approaches to corrective—and possibly preventive—strategies for these congenital abnormalities.

Congenital urinary tract obstruction, a condition termed uropathy, is one of the major causes of chronic kidney disease and ESRD in infants and children. The origins and best treatments of this disorder, however, are not well understood. To promote studies of these conditions, in March 2003, the NIDDK requested applications for basic and clinical research studies of obstructive uropathy. Among the goals of this effort are the development of objective prognostic markers; identification of genetic determinants of this congenital malformation; the development of reliable animal models of the malformation(s) to facilitate future research; and evaluation of the long-term effectiveness of various treatment strategies.

Pediatric Strategic Planning Task Force: As part of its planning process to guide future research areas of emphasis, in February 2005 the NIDDK sponsored a workshop that focused on assessing the "state of knowledge" and "future research needs" in areas related to pediatric urology. A multidisciplinary panel of clinical and basic science experts was convened to assess current scientific information related to pediatric urology and to develop suggestions for future research approaches. The topics for discussion in the workshop included many of the pediatric urological and nephrological developmental abnormalities, such as vesicoureteral reflux, ureteral abnormalities, and upper urinary tract obstruction.

It's Not the Shape, It's the Substance— NIDDK's Dr. Griffin Rodgers Offers Sickle Cell Update

For many years, doctors thought that the excruciating pain associated with sickle cell disease crisis was due to the sickled shape of the cell. The cells were contorted like sharp boomerangs rather than like friendly little disks and were thus tearing up the microcirculatory systems of patients.

In a recent talk at the NIH Clinical Center, NIDDK Deputy Director Dr. Griffin Rodgers described some of the key research advances and opportunities that can help to combat sickle cell disease, which in the U.S. occurs predominantly in people of African descent. For example, intramural research by investigators such as Drs. Connie Noguchi, Alan Schechter, William Eaton, and James Hofrichter at NIDDK helped to show that it is the chemical and biophysical properties of the hemoglobin within sickled cells, more than their shape, that impose the penalties of sickle cell disease. It is the accumulation of intracellular polymers, not the sickling of cells, that is principally responsible for pathogenesis. Dr. Rodgers holds out the hope of eventual stem cell or gene therapy as a cure for sickle cell disease, and illuminated gathering knowledge of the disorder, including recent studies by Schechter and his colleague Dr. Mark Gladwin, of the NIH Clinical Center, which implicate nitric oxide as a key contributor to the vascular constriction that is a hallmark of the disease.

"Sickle cell disease is one of the first diseases to be understood at the genetic level," said Rodgers. The disorder is caused by a single amino acid substitution and involves a reversible aggregation of sickle hemoglobin and eventual distortion of red blood cells, which can go back to their normal shape.

NIDDK Deputy Director Dr. Griffin P. Rodgers speaks at the NIH Clinical Center about NIH research into the causes of and treatments for sickle cell disease. Research supported by the NIH, and research conducted by NIH scientists at the Clinical Center, have contributed greatly to improvements in therapies for people with sickle cell disease.

There is a kind of parachuting effect that red cells undergo as they enter the microcirculation and erythrocytes loaded with polymer can't perform this function. Eventually, these cells can't traverse the microcirculation. Obstructions occur, affecting all organ systems. Rodgers called the disease's manifestations "protean" in that they involve neurologic complications, lung and liver ailments and periods of unrelenting bone and joint pain known as "musculoskeletal crisis."

Not every patient experiences the same level of severity; there are modifying factors owing to genetic and physiologic differences among patients. With respect to the former, scientists have been able to trace the migration of the sickle cell gene from the Old World to the New, more than 4,000 years ago. It appears to have originated in perhaps four sites in antiquity: Senegal (from which the least harmful condition emerged), Benin (home of an intermediate phenotype), India/Saudi Arabia (a mild, almost inconsequential version) and Bantu (associated with the most severe cases).

Several decades of NIH studies on fetal hemoglobin and its genetic control mechanisms have led to important discoveries. Dr. Rodgers and colleagues found that a cancer drug—hydroxyurea—can increase levels of fetal hemoglobin, thus moderating the disease's consequences. They launched the NIH Hydroxyurea Trial at the Clinical Center, in which patients remained at the hospital for up to three to four months while physicians tried escalating doses of the drug in a search for the optimal amount.

Most patients took two or three weeks to respond. While most experienced benefits, about 25-30 percent did not. Some began to feel better even before their fetal hemoglobin increased, so maybe there were other mechanisms at work.

In the 1990s, the National Heart, Lung, and Blood Institute funded a multicenter trial that was stopped early because, as a May 1995 article in the *New England Journal of Medicine* reported, there was a clear benefit to hydroxyurea therapy. It was associated with a 50 percent reduction in the frequency of painful sickle cell crisis, required less frequent blood transfusion, and reduced instances of "chest syndrome," a common cause of death in sickle cell patients. In 1998, the FDA approved hydroxyurea for use in sickle cell disease; it remains the only drug approved for that ailment. In 2003, the *Journal of the American Medical Association* published a nine-year follow-up study of patients taking hydroxy-urea. It showed that patients experienced continued increase in fetal hemoglobin levels, less acute chest syndrome occurrence and improved survival. The drug is now being actively studied in children with sickle cell disease.

Studies at the Clinical Center continue to refine the pathogenesis of hemolysis in sickle cell disease, and chemical factors affecting microvascular (or blood vessel) constriction. NIDDK's Schechter and Gladwin have shown how nitric oxide contributes to complications in the disease by regulating vasodilator tone and inhibiting platelet aggregation and adhesion, among other properties.

Dr. Rodgers envisions widening opportunities to intervene with drugs, and sketched the beginnings of an approach to a cure. Hematopoietic stem cell transplantation (HSCT) is one option researchers can pursue, but only one-quarter of patients have a suitable donor. Reduced intensity conditioning regimens followed by HSCT (non-myeloablative transplants) offer promise in adult patients in whom high doses of conventional preparative chemotherapy may prove unacceptably toxic. The Holy Grail is gene therapy, but unfortunately, that path is still a ways off. Improved methods are required to recognize true hematopoietic stem cells, to expand their number, and ultimately to have the replacement gene function in cells destined to become red cells. At the moment that's a difficult proposition. Cord blood might be useful as a source of hematopoietic stem cells for eventual gene therapy. Both the non-myeloablative and cord blood strategies are currently being pursued by Rodgers and his colleagues.

The NIH Clinical Center is an important venue for advances in sickle cell disease. The NIH will continue vigorous support of research toward a cure for this devastating disease.

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Sensing Calcium, Treating Disease

New treatments are emerging to correct abnormal calcium levels, which are common in the majority of patients who suffer life-threatening kidney disease and in people with certain rare diseases of the parathyroid glands, including parathyroid cancer. These treatments build upon substantial NIH investments in research that elucidated the role of the parathyroid glands in regulation of calcium levels. A new treatment strategy derives from the identification of the body's master regulator of blood calcium levels: the calcium-sensing receptor protein.

Because calcium is critical not only for bone formation but also for a myriad of other body functions, its levels are normally kept tightly controlled. In the 1960s and earlier, decades before the nature of the calcium-sensing receptor protein was defined, scientists studying large animal models found that low blood calcium levels cause the secretion of parathyroid hormone from parathyroid glands, while high blood calcium levels inhibit its release. Around 1960, NIDDK-supported scientists pioneered a method for preparing this hormone in a pure and stable form. They also developed what was then a new measuring technique, radioimmunoassay, to assay the very small quantities present in the human body. In the 1970s, these scientists devised a way to isolate cells from bovine parathyroid glands and grow them in the laboratory for study. These advances supplied the necessary tools for far more detailed experimentation.

From research supported by the NIDDK and others, scientists gained important insights into the regulation by calcium of parathyroid hormone secretion, the reciprocal influence of parathyroid hormone on calcium levels, and what happens when these processes go awry. Based on numerous studies over many years, scientists theorized that there is a calcium sensing mechanism on the surface of parathyroid cells that maintains constant surveillance of blood calcium levels. When calcium levels fall, this "calcium-sensing receptor" permits the secretion of parathyroid hormone, which then orchestrates a complex set of activities to help restore normal levels. These activities include the absorption of calcium from food in the intestines, its release from bones, and its reabsorption by the kidneys. When blood calcium levels become too high, the calcium-sensing receptor reins in parathyroid hormone. In diseases termed "hyperparathyroidism," this regulation is destroyed. Excess parathyroid hormone plunders the skeleton for its calcium, leaving bones more vulnerable to fracture and dumping potentially toxic amounts of calcium into the bloodstream. Patients may also experience fatigue, kidney stones, and impaired thinking.

In 1993, a group of scientists, funded in part by the NIDDK, identified the gene for the calcium-sensing receptor. Surprisingly, analysis of this gene revealed that the receptor is not, as previously thought, a channel through which calcium streams into cells. Rather, it is a novel member of a large family of proteins termed G protein-coupled cell-surface receptors. Because these proteins are prime drug targets for a number of health conditions, scientists had previously thought that the calcium-sensing receptor might be a good drug target for diseases marked by excess parathyroid hormone. Its landmark identification as a G protein-coupled receptor helped stimulate further research in this area.

With the gene for the calcium-sensing receptor in hand, scientists supported in part by the NIDDK discovered the underlying causes of some rare forms of "primary" hyperparathyroidism. People have two

copies of the calcium-sensing receptor gene. A mutation that reduces function of one copy causes reduced sensitivity to calcium in the parathyroids and kidney resulting in a mild, generally asymptomatic disorder termed familial hypocalciuric hypercalcemia (FHH). When mutations impair both gene copies, parathyroid cells are essentially totally unable to "sense" calcium, leading to a severe increase in secretion of parathyroid hormone. The resulting neonatal disease is severe, and removal of the parathyroids is required for babies to survive. This past year, researchers found that another form of hyperparathyroidism is an autoimmune disease: the body mistakenly produces antibodies that interfere with the calcium-sensing receptor's functioning. Other forms of primary hyperparathyroidism have been shown to result from excess—and sometimes cancerous—growth of parathyroid tissue.

Researchers have also, over many years, gained an understanding of "secondary" hyperparathyroidism, which is associated with kidney disease. When the kidneys fail, blood phosphate levels increase and calcium levels drop, as a result of loss of certain kidney functions important in calcium regulation, such as production of the active form of vitamin D, calcitriol. The body—in a doomed attempt to normalize calcium levels without healthy kidneys—then increases parathyroid hormone secretion. One result of secondary hyperparathyroidism is weakened bones, termed renal osteodystrophy in this context. Patients may also suffer from cardiovascular disease, likely related to disturbances in blood calcium and phosphate.

Treatments for hyperparathyroidism have not been ideal. Surgery to remove excess or abnormal parathyroid tissue has been, to date, the only effective way to treat primary hyperparathyroidism. Hyperparathyroidism related to kidney disease has been treated with phosphate binders, with calcium supplementation to increase its levels and thus suppress parathyroid hormone release, and by administering calcitriol, which also reduces the amount of parathyroid hormone. However, these therapies can result in high blood calcium levels and other problems. Surgery may then be necessary.

Research on the calcium-sensing receptor has now led to the development of a new drug by scientists at a biotechnology company. In 2004, investigators reported the results of an industry-sponsored clinical trial demonstrating the effectiveness of this oral drug in kidney disease patients on dialysis. It has been approved by the Food and Drug Administration for treating hyperparathyroidism associated with kidney disease. The drug is one of a novel class of compounds that interact with the calcium-sensing receptor in a way that "mimics" calcium. Called calcimimetics, they cause the receptor to perceive calcium levels in the blood as higher than they really are and thus reduce parathyroid hormone secretion. Calcimimetics, whose characterization was supported in part by the NIDDK, are also being explored for treating other forms of hyperparathyroidism, including high calcium levels resulting from parathyroid cancer.

Scientists are also currently investigating "calcilytics," compounds that have the opposite effect on the calcium-sensing receptor by leading to increased parathyroid hormone secretion. Paradoxically, parathyroid hormone can either weaken or help build bones—depending on the timing and extent of its secretion from the parathyroids. An orally-administered calcilytic may thus help treat osteoporosis by stimulating endogenous secretion of parathyroid hormone, obviating the need for injections of synthetic parathyroid hormone, recently approved by the FDA as a treatment to build bone.

NIH funding has contributed to a mosaic of vital advances, progressing from early basic research on parathyroid hormone and calcium regulation to the recent development of a new drug. Collectively, these discoveries represent a striking example of "translational" research, in which both NIH- and industry-supported investigators have benefited patients by propelling science from the bench to the bedside.

Jill Khederian

Fighting a Constant Battle Against Kidney Stones

Every time Jill Khederian leaves her home, she scans the horizon for the nearest restroom. "I have to drink so much water that every time I turn around I can pretty much tell you where any bathroom is," says the 53-year-old former post-anesthesia recovery room nurse and mother of three. Jill suffers from a painful and chronic condition called cystinuria, a rare, inherited disease that causes cystine stones to form over and over again in her kidneys. Cystine stones are difficult to treat and require life-long therapy. Drinking three to four liters of water a day helps prevent the creation of the stones, and when stones do form, helps to pass them through the body. But Jill's story is much more complicated than simply drinking lots of water to manage her disease. Left untreated, her condition could lead to end-stage kidney (renal) disease, requiring renal dialysis or a kidney transplant to survive.

Jill Khederian with her Jack Russell terrier, Sonya.

About Kidney Stones

Patients with kidney stones can experience one of the most painful of all disorders. Kidney stones affect the urinary tract. The urinary tract, or system, consists of the kidneys, ureters, bladder, and urethra. The kidneys are two bean-shaped organs located below the ribs toward the middle of the back. The kidneys remove extra water and wastes from the blood, converting them to urine. They also keep a stable balance of salts and other substances in the blood, and produce hormones that help build strong bones and help form red blood cells. Narrow tubes called ureters carry urine from the kidneys to the bladder, which expands to store the urine until it is emptied through the urethra to outside the body.

Kidney stones are hard masses that develop from crystals that separate from the urine as it is forming and build up on the inner surfaces of the kidney. Kidney stones are unrelated to gallstones, which form in a different area of the body. There are several different types of kidney stones, including calcium (the most common type), uric acid, struvite, and cystine. Urine contains chemicals that can normally prevent some types of crystals, or stones, from forming. These chemical inhibitors, however, do not seem to work for everyone, or they can be overwhelmed when there is an excess of a stone-forming agent in the urine. The good news is that most kidney stones pass out of the body without any intervention by a physician. However, the passing of a stone can be excruciatingly painful.

PATIENT PROFILE

Stones that are three millimeters or larger usually require more intensive treatments, including extracorporeal shock wave lithotripsy, or ESWL, the most frequently used therapy. In ESWL, shock-waves that are created outside the body travel through the skin and body tissues until they hit the stones, which are denser than the tissues. The stones break down into sand-like particles that are easily excreted in the urine. For stones that are quite large or in a location that does not permit effective use of ESWL, percutaneous nephrolithotomy may be recommended. In this procedure, the surgeon makes a tiny incision in the patient's back and creates a tunnel directly into the kidney to remove the stone. The least invasive treatments, however, are always the most preferred.

Kidney stones are not rare; they are one of the most common disorders of the urinary tract. An estimated 5 percent of adults in the United States have reported ever having a kidney stone, and men tend to be affected more frequently than women.

Many Americans may pass one kidney stone in their lives, but others may suffer from recurring, painful stones. Once a person gets more than one stone, others are likely to develop. The underlying cause for an individual kidney stone can vary from excess vitamin D in the diet to one of several metabolic disorders, and in some cases may remain unknown. While some causes of recurring stones are known, such as the inherited diseases cystinuria and hyperoxaluria (which causes calcium-oxalate stones), researchers are still teasing out what causes other patients to become highly susceptible to forming stones. A better understanding of the interplay between the diet, genetic predisposition, and metabolic dysfunctions that leads to the formation of kidney stones will also improve treatment and provide clues to prevention.

Living with Cystinuria

Cystine is an amino acid that does not dissolve well. Cystine is actually a special "di-amino acid" found in a number of proteins, in which it acts as a stabilizing unit. Small amounts of cystine and other amino acids enter the blood from the diet and from protein metabolism. When blood is filtered through the kidneys to remove wastes, cystine and the other amino acids get filtered, too. Normally, the kidneys reabsorb amino acids and other non-waste molecules while urine is being formed. In Jill and other people with cystinuria, the disease gene causes high levels of cystine in the urine. The excess cystine forms crystals that develop into cystine stones. The danger of not being able to pass these stones is that they can block the flow of urine, causing ongoing urinary tract infection and damage to kidney tissues, as well as cause constant bleeding. Trying to prevent cystine stones from developing is important because medical management is less successful with cystine stones than with more common types of kidney stones. However, prevention is more easily said than done.

In 1970, Jill was a 19-year-old college sophomore when she passed her first stone. "I was living off campus with a friend when I began feeling this pain," says Jill. The pain began as an ache in her back and side. Then, it became more constant and severe as her urinary system tried to rid itself of the stone. She experienced a burning sensation during urination and blood in her urine, as well as a frequent urge to urinate. She became nauseated and began vomiting, and her lower abdomen was painful when touched. Jill, who has passed several stones since, describes the pain as "like you've been hit by a car," a gripping pain that even painkillers can barely ameliorate. "I've given birth to three children, all [without drugs]" she says, "and that doesn't even compare to passing a kidney stone."

The most common symptoms of kidney stones include:

- **Extreme, sharp pain in the back or side that will not go away**
- **Blood in the urine**
- **Nausea and vomiting**
- **Cloudy or odorous urine**
- **Frequent urination**
- **A burning feeling when you urinate**
- **Fever and chills**

The stone Jill passed at age 19 was found to be 100 percent cystine. Subsequently, she was diagnosed with cystinuria. Neither of her parents suffers from the disease; her father has passed a couple of kidney stones, but they were calcium stones. Yet, she has learned that both parents are carriers of the gene that causes cystinuria. Jill's older brother, John, also has the disease. Because both biological parents need to carry the gene to pass the disease on to their children, Jill's three daughters most likely will be spared from developing cystine stones because their father, Jill's husband, is not a carrier.

Treating Cystinuria

Jill underwent her first major surgery in 1977 when a stone obstructed one of her ureters, which are the tubes that conduct urine from the kidneys to the bladder. As a result of the blockage, her left kidney began to swell. "I was told I needed a left pyelolithotomy, a surgical procedure to remove the stone, or I might lose a kidney and possibly die!" says Jill. Even major surgery failed to remove the entire stone. "Because of its location and hardness, physicians were able to remove most, but not all of it," adds Jill.

Despite their small size, recurring kidney stones can pose tremendous difficulties for patients, as Jill has found. Over the years, she has taken several different medications to try to prevent cystine stones from developing in her kidneys. Some have resulted in side effects, including skin rashes and swollen joints in her fingers. She is currently on 1,200 milligrams a day of tiopronin. This drug changes the chemical composition of cystine, and is prescribed to prevent cystine stones from forming. Unfortunately, the drug has a relatively short shelf life, is expensive, and—like many medications—has potentially severe side effects that need to be monitored by a physician. Jill has also undergone serious medical procedures for her condition over the past five years, including three laser surgeries, one of which resulted in a three-day hospital stay.

Research on Kidney Stones

While there are multiple causes and types of kidney stones, the end result is the same: an imbalance in urine components that leads to a painful precipitate that can damage the kidneys and harm urologic function. Researchers are working on finding and developing new drugs with fewer side effects to prevent or treat kidney stones. In addition, the growing field of lithotripsy has greatly improved the treatment of kidney stones. However, researchers continue to seek answers to questions such as:

- Why do some people continue to have painful stones?
- How can doctors predict, or screen, those at risk for getting stones?
- What are the long-term effects of lithotripsy?
- What role do genes play in stone formation?
- What other natural substance(s) found in urine can block stone formation?

PATIENT PROFILE

Cystinuria is a lifelong condition causing recurrent kidney stones. The rate of stone formation can sometimes ease up as patients get older. Unfortunately, Jill has not experienced symptom reduction, which makes prevention even more important for her. Morever, she is very concerned that she might follow in her brother John's footsteps. He underwent an operation in the 1980s that uncovered hundreds of cystine stones in his kidneys. Like a number of kidney stone sufferers, he has experienced serious kidney complications. "My brother is four years older than I am. He has high cholesterol, high blood pressure, permanent damage to his left kidney, and he just recently developed anemia, all of which is believed to be related to his cystinuria. He's been told that within a year it's very likely he will need dialysis," Jill says.

Fortunately for Jill, right now her blood pressure is low to normal; her cholesterol is fine; and she shows no signs of kidney failure. To stay in shape, Jill exercises regularly. And to do what she can to prevent the recurrence of her cystine stones, she flushes her system with plenty of water. "I'm always aware of my need to be drinking water," she says.

Many of the advances in kidney stone treatments, including those for cystinuria, have resulted directly from NIDDK investments in research to understand the underlying causes of kidney stone disease. Translation of basic understanding of kidney stone disorders into applicable treatments or cures is the ultimate goal for research in this area. The NIDDK continues to strengthen its research program on the causes and potential treatments for recurring kidney stones. For example, recurring calcium oxalate stones, the most common form of kidney stones, are caused by inherited metabolic diseases such as the primary hyperoxalurias, but more commonly occur "spontaneously"—though with some familial tendency. It is suspected in the latter case that there are unique genetic, environmental, and/or metabolic factors that predispose individuals to recurrent calcium oxalate stone formation. A current NIDDK initiative is encouraging innovative research on all forms of calcium oxalate stone disease in an effort to shed light on pertinent metabolic, molecular, and genetic defects, and to eventually develop more effective diagnostic, treatment, and preventive strategies. Studies to determine the contribution of genetic factors to kidney stone formation in the general population will also be helped by information and research tools evolving from the NIH investment in the Human Genome Project. The Institute is also pursuing a research agenda in hereditary calcium oxalate stone disease, in order to address the special needs of this patient group.

The National Kidney Disease Education Program (NKDEP)

An estimated 10 to 20 million Americans currently suffer from kidney damage, also called chronic kidney disease. Each year, over 300,000 must have kidney dialysis to stay alive. The number of people developing irreversible kidney failure, also called end-stage renal disease (ESRD), has doubled each decade for the last two decades, and disease statistics indicate that this trend is likely to continue. The leading causes of kidney disease are diabetes and high blood pressure. If current trends continue, the recent increases in obesity and type 2 diabetes in the U.S. will have grave implications, as more and more people develop kidney complications related to diabetes. The public and private costs of treating ESRD were estimated at $23 billion in 2001.

Fortunately, chronic kidney disease can be slowed, if not prevented, provided it is detected early. Good control of blood sugar and blood pressure can reduce the risk of developing kidney disease. Diets low in protein can also slow kidney disease progression. In spite of these advances in treatment and prevention, only a small number of people who most need proper screening or treatment receive it. The NKDEP strives to disseminate information on prevention and treatment to physicians and patients who can most benefit from it.

Racial and ethnic minorities suffer a far greater incidence and prevalence of irreversible kidney failure than Caucasians. Rates of ESRD are disproportionately greater in African Americans, American Indians and Alaska Natives, Native Hawaiians and other Pacific Islanders, and Hispanic Americans. Diabetic kidney disease is the most common cause of ESRD in all of the minority groups except for African Americans, in whom high blood pressure-induced kidney damage is also a major cause.

The ultimate goal of the National Kidney Disease Education Program (NKDEP) is to reduce complications and death due to kidney disease and kidney failure among all Americans. Currently, the NKDEP is targeting primary care providers and people at high risk for kidney disease—particularly African Americans with diabetes, high blood pressure, or a family history of kidney failure. In June 2004, the NKDEP nationally launched the campaign, "You Have the Power to Prevent Kidney Disease," which emphasizes three key messages:

- Early detection is important. If you are at risk due to diabetes, hypertension or a family history of kidney failure, talk to your doctor about having your kidneys checked.
- Effective treatment can prevent or slow kidney damage.
- Left undiagnosed and untreated, kidney disease can lead to kidney failure.

The program plans to broaden its target audiences to include other at-risk audiences as the program expands. Prior to launching the national campaign, NKDEP piloted the program in four cities: Atlanta, GA; Baltimore, MD; Cleveland, OH; and Jackson, MS. Successful strategies identified through the pilot sites were used to inform the national campaign.

In addition to public awareness activities, the NKDEP has several Work Groups that are striving to remove specific barriers to better kidney disease awareness and care. The membership of these groups is drawn from the professional partnership network of the NKDEP, which includes non-profit groups, industry, and government. The NKDEP Laboratory Work Group has made efforts

to encourage improvement and standardization of the serum creatinine assay–which is used to estimate how well the kidneys are functioning—in order to address issues of inter-laboratory variation in this assay. The group has also begun efforts to encourage laboratories to report glomerular filtration rate (GFR) estimates as soon as possible in adults with low GFRs, to enable physicians to quickly identify individuals with impaired kidney function. The NKDEP Quality Indicators Working Group, in partnership with the Centers for Medicare and Medicaid Services (CMS), is undertaking a pilot project to spur the development of quality indicators of care for chronic kidney disease among Medicare beneficiaries hospitalized for cardiovascular disease.

Through all of these efforts, the NKDEP is a positive force in helping to reduce the burden of kidney disease in the U.S.

Molecular Basis of Urinary Tract Infections: More to the Picture than Meets the Eye

Dr. Scott J. Hultgren

The NIDDK National Advisory Council meets three times annually to provide advice to the Institute regarding its research portfolio and broad issues of science policy. These meetings are also an opportunity for the Council members to learn about recent scientific advances in different fields through presentations from NIDDK-supported extramural scientists. In September 2004, the Council and NIDDK staff were privileged to hear from Dr. Scott J. Hultgren, a leading researcher in the field of bacterial pathogenesis. Dr. Hultgren is the Helen L. Stoever Professor of Molecular Microbiology at the Washington University School of Medicine in St. Louis, Missouri. He received his Ph.D. at Northwestern University in Chicago in 1987, and conducted his postdoctoral studies at Umeå University in Sweden until 1989. Dr. Hultgren's research team is pushing to decipher the molecular crosstalk that takes place between pathogenic bacteria and cells in the bladder and to translate this information into possible therapies for recurrent urinary tract infections. The following highlights are adapted from his presentation to the Council.

Like marauders at the castle gates, microbes seeking to colonize a host must battle and prevail against host defenses before they can reach a safe haven. Stealth, timing, and complex biological activities at the cellular level all come into play. In the case of bacteria that colonize the bladder and cause urinary tract infections, exciting results are emerging from in-depth study of these activities at the host-pathogen interface. Not only do these bacteria activate myriad innate host defense systems, but recent studies also suggest that one result of infection is bladder cell turnover and proliferation—an observation that may, in turn, provide insight into pathways important in bladder cancer. Moreover, molecular pathways are activated in the bacterium upon its interaction with the host. These activated pathways enable the bacterium to subvert innate host defenses, persist in the urinary tract, and cause disease. UTIs are quite prevalent: an estimated 34 percent of adults, mostly women, have had at least one urinary tract infection. Understanding both bacterial tricks and host defenses in bladder infections may ultimately result in new and more effective treatments for these infections, prevent their recurrence, and uncover important aspects of bladder biology.

A Sticky Pike: The Pilus

Escherichia coli, or *E. coli*, is a rod-shaped bacterium normally found in the colon, where it aids the body in the last stages of digestion. However, some *E. coli* acquire features that significantly enhance their ability to survive and cause disease if they are accidentally introduced into the urinary tract. These strains of bacteria are called uropathogenic *E. coli*, or "UPEC."

Most bacteria that reach the bladder will get flushed out with the urine. UPEC, however, use hair-like fibers with sticky tips, called pili, to adhere firmly to cells lining the bladder. While pathogenic *E. coli* may have more than one type of pilus on their surfaces, the pilus important for UPEC attachment to bladder

cells is called the "type 1" pilus. From studies spanning a number of years, a detailed molecular picture has emerged of how the type 1 pilus and similar adhesive pili are constructed. This knowledge has provided insight into the strength of these structures so important to UPEC pathogenesis, and into ways to disrupt their assembly.

In a series of steps called the "chaperone-usher" pathway, bacteria assemble the type 1 pilus and similar pili from an array of pilus subunit proteins. The role of the "chaperone" protein in this bacterial pathway is to grab the immature pilus subunits and prepare them for assembly into a complete pilus. To carry out its role, the chaperone employs an unusual molecular tactic: after making contact with a pilus subunit, the chaperone guides the subunit to assume its proper three-dimensional structure by temporarily providing it with a missing piece that completes the structure. Chaperone-subunit complexes then move to a pilus assembly site at the bacterium's outer membrane, known as an "usher." As the pilus subunits are assembled, they interlock like Lego™ pieces—each subunit now providing to the next the missing structural element originally supplied by the chaperone. The nascent pilus is channeled through the usher to the surface of the microbe, where it can assume its final, functional conformation, which is very stable and strong.

A key finding with potential implications for anti-microbial, including anti-UPEC, therapy has emerged from this elucidation of pilus assembly. The chaperone-usher pathway is shared by *E. coli* and many other species of mostly disease-causing bacteria, ranging from those that cause plague to those that cause urinary tract infections. In the hundreds of bacterial species that have been studied, the chaperone proteins all possess a conserved molecular site in the part of their structure that first contacts the pilus subunits. This site is crucial to the chaperone's ability to interact with the subunits. If this site is altered, the pilus subunits cannot be assembled and they degrade. The importance of this site to chaperone activity and pilus assembly, as well as its conservation among so many species, makes it an attractive target for therapy. Currently, Dr. Hultgren's research team is working to design a drug that would bind to this site in the chaperones and inhibit the assembly of adhesive pili—thereby severely diminishing the capacity of piliated bacteria to cling to and colonize host tissues, such as the bladder. Such a drug could have broad spectrum activity against a wide range of pathogenic bacteria.

Bladder as Battlefield

When UPEC reach the bladder, they meet a complex organ. The bladder is composed of several layers of epithelial cells, connective tissue, and muscle. The cell layers most involved in interactions with UPEC, however, are the superficial umbrella cells—which constitute the top inner layer of the bladder—and the intermediate, or transitional, epithelial cells, which rest on the basal cells. The superficial umbrella cells are terminally differentiated, meaning that they have attained a specialized functional state and can no longer proliferate. However, the underlying transitional cells are less mature and are responsible for renewing these specialized cells as needed.

The superficial umbrella cells are perhaps some of the largest cells in the body, and they produce very interesting proteins called uroplakins. Studies of these proteins by another NIDDK-supported research team have shown that there are four uroplakins, which assemble in groups of six into hexagonal structures with a central "pore." The hexagonal particles are then further organized to form a crystalline plaque-like material with a dense honeycomb appearance, which coats the surface of the bladder. This uroplakin coating forms an impermeable surface to prevent the leakage of toxic molecules from the urine into the bladder epithelium.

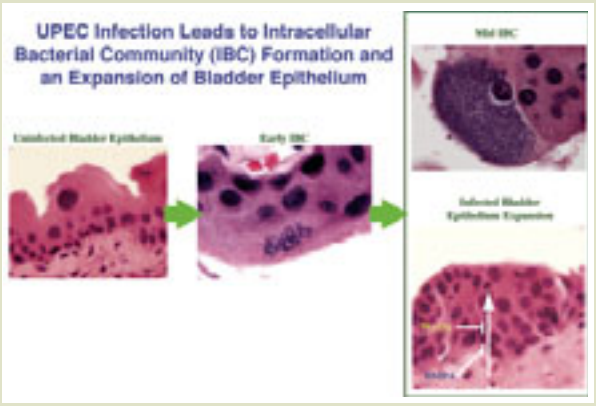
Yet, while uroplakins are meant to be a shield, in the case of attack by UPEC, they are also an Achilles heel. UPEC elaborate type 1 pili that are able to

bind to a specific sugar—mannose—that is present on the uroplakins. A protein present at the tip of the type 1 pilus, called FimH, is key to recognizing the mannose molecule. Using high resolution electron microscopy, Dr. Hultgren's research team has observed the tips of the type 1 pili fibers buried within the mannose-rich central pore of the uroplakin particles. This finding confirms visually other experimental data that suggested that interaction between the type 1 pilus adhesive tip and the uroplakin proteins is the initial step in UPEC attachment to bladder cells.

UPEC attachment is met with firm resistance, however. One of the most dramatic consequences of this host-pathogen interaction is that it activates a pre-programmed, self-destruct sequence in the superficial umbrella cells. Activation of this mechanism leads the affected cells to slough off the inside of the bladder wall in about 12 hours. Other changes occur, too. Normally, the bladder is very quiescent, and regeneration of bladder tissue is very slow, occurring only maybe every six months to a year or so. But upon UPEC infection, normally silent regeneration pathways are activated within hours, allowing the underlying transitional cells to proliferate and differentiate to replace the exfoliated superficial umbrella cells. This process likely represents a very potent innate defense; by exfoliating and replacing the exposed superficial umbrella cells, the bladder can rid itself of contaminated cells with minimal tissue injury. Finally, assault by UPEC triggers the bladder cells to signal for assistance from immune system cells, called neutrophils, which flood into the bladder to fight the bacteria. The bacteria thus need to subvert these defenses in order to survive in the urinary tract.

Differentiation in Host Defense

Building upon the knowledge about the interaction between the FimH protein and uroplakins, Dr. Hultgren's research team was able to probe beneath the surface to find out what happens within the bladder cells that drives their renewal in reaction to UPEC colonization. One experiment looked for genes that were turned



Consequences of bladder infection by pathogenic *E. coli* bacteria. Left panel, uninfected bladder cells stained pink with purple nuclei; center panel, bladder cells (light purple with dark purple nuclei) with an early infection of tiny dark blue bacteria; right panel, bottom, proliferating bladder cells stained light pink with dark purple nuclei; right panel, top, a bacterial network visible within a single bladder cell as tiny dark blue specks to the left of the cell's nucleus.

"on" or "off" in mouse bladder cells when mice were infected with two different laboratory strains of UPEC. One strain had a normal FimH, and the other lacked FimH and could no longer adhere to mannose. This experiment revealed about 50 genes that are differentially regulated in the bladder cells infected with the normal UPEC. Many of the genes identified are involved in pathways governing cellular differentiation and proliferation. Two of the cellular pathways that came to light from this study are the "BMP4 pathway" and the "sonic hedgehog" pathway.

The BMP4 pathway is a very complicated cellular pathway that, when activated, leads to signaling cascades that ultimately inhibit cellular differentiation in many developmental systems. Results from two different types of experiments indicate that UPEC infection induces down-regulation of this pathway within a few hours, whereas infection with the FimH mutant UPEC has no effect. These results suggest that the BMP4 pathway normally acts to suppress unnecessary differentiation of transitional cells, but is deactivated upon UPEC infection in order to allow

the regeneration processes it governs to be activated. Similarly, a few hours after infection, sonic hedgehog—an activator of development—is up-regulated in bladder cells, potentially promoting regeneration. These tantalizing results have launched a new set of studies to capture the molecular crosstalk at the host-pathogen interface and determine the precise molecular mechanism(s) by which UPEC infection activates proliferative pathways and represses inhibitors of proliferation.

These findings may also shed light on growth pathways involved in bladder cancer. Infections with some species of bacteria, such as the *Helicobacter pylori* bacteria that are associated with stomach ulcers, have been implicated as predisposing factors for certain cancers. Analysis of patients with bladder carcinoma has revealed that 60 percent of their tumors have deletions in a specific region of chromosome 9. This finding suggests that genes encoding so-called "tumor suppressors" exist in this region of the chromosome. Interestingly, a gene encoding one of the components of the sonic hedgehog pathway is found within the same region of chromosome 9. Thus, study of cellular proliferation pathways activated in response to UPEC infection in animal models may also uncover heretofore unrecognized connections between bacterial infection and bladder diseases, such as bladder cancer, that are associated with dysfunctional regulation of cellular growth and differentiation—or, simply provide a tool to identify the relevant pathways. Studies are under way to investigate in mouse models whether deleting the genes identified in the normal and FimH mutant UPEC-infected mouse screen will lead to bladder tumor formation.

Invasion and Evasion by *E. coli*

The host pathways that are activated when UPEC interact with bladder tissue represent potent innate defenses. So the question remains, why is urinary tract infection such a problem in the clinic? It appears that the bacteria have evolved mechanisms to subvert these innate defenses so that they can persist and cause disease.

While the interaction between type 1 pili and a bladder cell triggers host defenses, another consequence of attachment is that it occasionally triggers the "zippering" of the superficial umbrella cell around the attached UPEC bacterium—a desirable event from the bacterium's perspective. Once inside the cell, the successful bacterium starts a race against time to ensure its survival and eventual spread to new host cells. UPEC can actually invade both superficial umbrella cells and the underlying transitional cells; however, they undergo an unusual growth pattern in the former, and an intriguing series of events begins to unfold. These events have been captured in "real time" using time-lapse video microscopy to film UPEC-infected cells in mouse bladders over the course of infection.

After entering superficial umbrella cells, UPEC proliferate rapidly, transitioning from a single rod-shaped bacterium to a developmental stage called an early "intracellular bacterial community" (IBC). These early IBCs are loose collections or "clumps" of typically rod-shaped *E. coli*. After about 6 hours, however, the UPEC in these early communities change dramatically, transitioning to a mature "mid-IBC" stage. In this stage, bacterial growth slows, the bacteria become rounded, and the chaotic clump becomes a dense, organized population embedded in a matrix of polysaccharides (chains of sugar molecules). This fascinating development and its similarity to a unique type of bacterial growth led the mid-IBCs to be dubbed "biofilm-like" networks. In mice, the mid-IBCs—filled with up to one million UPEC—actually press the surfaces of their host cells outward and can be seen as uroplakin coated "pods" on the inner surface of the bladder. Finally, a few hours after the mid-IBCs form, the bacteria undergo another change, in which they start to detach from the network, become motile, and "flux" out of the host cell to reinitiate the invasion cycle in other bladder cells.

The organized bacterial networks formed by the mid-IBCs are especially interesting—and enlightening—due to their similarity to biofilms. Biofilms are special bacterial communities often associated with bacterial

persistence in a variety of environments, including diseased human tissues and medical devices. They are particularly resistant to antibiotics and to host immune responses. This resistance is thought to be facilitated by two key features of biofilms: the formation of the protective polysaccharide matrix and the development of "community behavior." Through the latter, the bacteria work together to sense and react to the environment, both to protect themselves and to enable spread into new environments. As a biofilm matures, the bacteria often form distinguishable subpopulations that are thought to serve distinct functional purposes in this community, similar to bees in a hive. Variation in the population also enhances the likelihood that at least part of the population could survive changes in the environment.

The observed generation of intracellular biofilm-like communities during UPEC infection has raised a number of questions, such as, what are the critical molecular signals controlling the maturation event? And, what signals tell the bacteria to stay put in the IBC matrix, and then disperse at the right time and in a directed, orderly fashion? There are a number of hypotheses about host signals that are involved, as well as signals elaborated by the bacteria that may enable them to work and communicate with each other in the IBC. For example, Dr. Hultgren's research team hypothesized that genes involved in pathogenesis—such as those important for making the type 1 pilus—are likely to be involved in the IBC activities. The team conducted experiments to analyze gene expression from the genetic element that controls the genes for the type 1 pilus subunits. The results of these experiments suggest that expression of the type 1 pilus genes increases over time in bacteria within the IBCs. This view was confirmed by using high resolution microscopy to peek inside the pods, which revealed that every bacterium is entirely coated with fibers, many of which appear to be type 1 pili. Interestingly, the fibers are not so much interacting with other bacteria, as much as they are interacting with the surrounding matrix. This interaction may potentially help to organize the bacterial network in its very defined biofilm-like array—and thus, possibly fulfill multiple roles in UPEC pathogenesis.

Ultimately, to understand the behavior of the biofilm-like IBCs, it will be important to be able to capture and understand the gene expression profile for each bacterium in the biofilm. Dr. Hultgren's research team is developing techniques to determine, for example, which other bacterial genes are also "on" or "off" during type 1 pilus gene expression. Another interesting aspect of these studies will be to discover the role of these IBCs in generating diversity in fitness—that is, how well they prepare the UPEC so that, as the bacteria emerge from the network and host cell, they are more fit to deal with stresses and to spread in the urinary tract and in the environment.

One interesting adaptation by UPEC to the hostile environment of the inflamed bladder is filamentation. When the late-IBC UPEC begin to flux from the host cell, many return to their normal rod shape. Some, however, develop into tremendously long rods—sometimes over 20 times their normal length. These filamentous bacteria are apparently impervious to attack by the immune system neutrophils, but still competent to invade new host cells. Already stymied by the protective, uroplakin-coated IBC pods, the neutrophils are thus further handicapped in their ability to clear the UPEC infection. Thus, the IBC pathway is both a protective and an adaptive response used by the bacteria in their quest for survival in the bladder.

Lurking Bacteria

After second and third generations of IBC formation, something interesting happens. The intracellular replication stops. The bacteria, after about two weeks, stop dividing altogether. They no longer replicate and no longer form biofilm-like intracellular communities. However, bacteria can co-exist in a cell and persist for months in this quiescent state.

Observation of this curious phenomenon has led to a key hypothesis: that the quiescent bacteria comprise a reservoir that may explain recurrent urinary tract infections in humans. In humans, a key diagnostic tool for UTIs is the presence of cultivatable bacteria

in the urine. Absence of bacteria in the urine following antibiotic treatment is thought to indicate successful clearance of infection. However, the urine of mice that are harboring quiescent UPEC bacteria is also sterile—despite the fact that their bladders are infected. If what is true of the mouse model is also true in humans, a lack of bacteria in the urine might not necessarily mean a lack of bacteria in the bladder. Thus, the urine culture may actually be missing bacteria lurking in the bladder epithelial cells, awaiting a signal to emerge and reinitiate an active infection.

If UPEC can exist in a quiescent state in human bladders and through this route are indeed responsible for some portion of recurrent UTIs, one possible trigger for their reactivation could be the regeneration of the cells lining the bladder. This regeneration requires the underlying cells to start proliferating. Experiments have revealed that the UPEC not only like to form IBCs in superficial umbrella cells, but that they will also form them in underlying cells that have been artificially stimulated to proliferate—cells that normally do not support IBC formation. Thus, these observations have laid the groundwork for other studies seeking to determine the clinical relevance of intracellular UPEC.

Translating from the Laboratory Bench to the Bedside and Back Again

The current clinical paradigm for recurring UTIs is that *E. coli* is an extracellular pathogen in the urinary tract and that recurrence of infection is always due to a "fresh" inoculation from an external source, such as the colon. The preceding findings suggest a very different paradigm for urinary tract infections, in which invasion of bladder cells by UPEC is critical to the ability of these bacteria to persist in the bladder, cause disease, and quite possibly to cause recurrent infections. Dr. Hultgren, his research team, and collaborators are pursuing the clinical relevance of these findings. In a current study, women who have had at least one recurrence of a UTI are being monitored regularly for bacterial load in the urine and for whether they have a recurrence. UPEC isolated from these women are being inoculated into mouse strains to determine whether they form IBCs. At the same time, the clinically isolated bacteria are being examined to pinpoint genetic differences among them that are associated with differences in their ability to form IBCs or with variations in UTI symptoms. Thus, applying knowledge gained from a fundamental understanding of how UPEC interact with an animal host to the analysis of actual clinical infections may translate into both a better understanding of UPEC pathogenesis and improved treatments for UTIs.

Hypotheses to Pursue

So far, much has been discovered about the structural basis of the initial host-pathogen interaction, how that leads to the activation of the IBC developmental pathway, and how these intracellular communities are the home for generating diversity in the UPEC bacterial populations so that when they flux out of the host cells, they are possibly more fit to colonize the urinary tract as well as the environment. The relevance of the UPEC reservoir in bladder cells to recurrent UTIs remains a key clinical question. Clues as to the signal(s) for the reactivation of these reservoirs back into acute infections with rapid growth and IBC formation are beginning to emerge. Finally, understanding the overlap between infection and regeneration processes, and how that may be important in not only normal bladder development, but abnormal bladder development, such as bladder cancer, is an important avenue to pursue.

In conclusion, the work in the Hultgren lab seeks to understand the molecular details of each step of an encounter between a pathogenic bacterium and its host tissue. Dr. Hultgren's research program integrates multiple disciplines, ranging from innovative translational research to providing snapshots of molecules caught in the act of triggering disease processes. His work is leading to a better understanding of UPEC infection, sparking better therapies to treat chronic and recurrent infections, and generating models valuable for the study of an array of human diseases.